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ORIGINAL ARTICLE

Synthesis, characterization and DNA cleavage studies of isomeric pyridyl-tetrazole ligands and their Ni(II) and Zn(II) complexes

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Abstract A new series of Ni(II) and Zn(II) complexes were synthesized from bidentate isomeric pyridyl tetrazole ligands such as 2-(1-vinyl-1H-tetrazol-5-yl)pyridine ($\mathbf{L^1}$), N,N-dimethyl-3-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)propan-1-amine($\mathbf{L^2}$), 2-(2-vinyl-2H-tetrazol-5-yl)pyridine($\mathbf{L^3}$), N,N-dimethyl-3-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)propan-1-amine ($\mathbf{L^4}$). All the complexes were characterized by the elemental analysis, molar conductance, FTIR, UV–vis and magnetic studies. The conductance and spectroscopic data suggested that, the ligands act as monobasic bidentate ligands and form octahedral complexes with general formula $[M(\mathbf{L^{1-4}})_2Cl_2]$, (M = Ni(II) and Zn(II)). In addition metal complexes displayed good antioxidant and moderate nematicidal activities. The cytotoxicity of ligands and their metal complexes have been evaluated by MTT assay. The DNA cleavage activity of the metal complexes was performed using agarose gel electrophoresis in the presence and absence of oxidant H_2O_2. All metal complexes showed significant nuclease activity in the presence of H_2O_2.

1. Introduction

Biologically active isomeric pyridyl tetrazole derivatives have been under great investigations as part of inorganic chemistry. Polyazole rings are versatile ligands [1] for coordinating transition metals, therefore synthesis of transition metal complexes containing polyazole rings, particularly tetrazoles and their derivatives has been given enormous significance, due to their practical applications [2–4]. There is an increasing interest of tetrazole derivatives for the development of ''click'' chemistry

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which was reported by Sharpless and co-workers [5]. Conversely, tetrazole-based compounds have made known special functionalities with interesting structures [6]. Tetrazole derivatives have found applications in therapeutics as antihypertensive agents [7], antibiotics [8] and drugs for AIDS treatment [9].

Even though many tetrazole containing derivatives are available in the literature, there is always an increasing demand for the development of novel and effective tetrazole containing therapeutic agents. In continuation of our ongoing research on DNA binding and cleavage activities of transition metal complexes [10], in this paper we presented the synthesis, characterization and DNA cleavage activities of Ni(II) and Zn(II) complexes which are obtained by the reaction of pyridyl-tetrazole derivatives which contain pendant arms like vinyl or propyl-$N(CH_3)_2$ group.

2. Experimental materials and measurement

Chemicals were purchased from Sigma–Aldrich and metal salts used in the preparation of the complexes are of reagent grade. The solvents used in the synthesis of the ligands and metal complexes were distilled before use. All other chemicals were of AR grade and were used without further purification. The elemental analysis of carbon, hydrogen, and nitrogen contents was performed using Perkin Elmer CHNS analyzer. Molar conductance of the complexes was measured using a Digisun conductivity metre in DMF. The electronic absorption spectra of the complexes were recorded on a JASCO V-670 Spectrophotometer in the wavelength region of 250–1400 nm in the solid state. The FTIR spectra of the complexes were recorded on a Tensor 2 FTIR spectrophotometer in the region of 4000–400 cm^{-1} using KBr disc. The magnetic susceptibilities of Ni(II) complexes were measured with a Sherwood scientific balance. Diamagnetic corrections were calculated from Pascal's constants. The magnetic moment values were calculated using the relation $\mu_{eff} = 2.83\ (\chi mT)1/2$ B.M.

2.1. Synthesis of ligands

2.1.1. Ligands

The preparation of **L** is carried as per literature [11] M.p. 221–223 °C. C, 48.98; H, 3.43; N, 47.60, ^{1}H NMR (CD_3OD): 8.56 (d, 1H, J = 7.9 Hz, pyr-H), 8.0 (d, 1H, J = 7.8 Hz, pyr-H), 7.79 (t, 1H, J = 7.8 Hz, pyr-H), 7.26 (t, 1H, J = 7.9 Hz, pyr-H), 7.1 (s, 1H, tetrazole-H) ppm.

2.1.1.1. N,N-dimethyl-3-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)-propan-1-amine(L^2) and N,N-dimethyl-3-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)propan-1-amine (L^4). To a solution of compound **L** (1.0 g, 6.8 mmol) in DMF (30 mL) was added potassium carbonate (4.6 g, 33 mmol) followed by 3-chloro-*N,N*-dimethyl propan-1-amine. HCl (2.1 g, 13.6 mmol). The reaction mixture was stirred at 70 °C for 50 h. After cooling, the insoluble material was filtered, the solvent was removed under reduced pressure, the crude mass was diluted with EtOAc (50 mL) and washed successively with water (3 x 40 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford a crude oil, which was purified by column chromatography on silica gel (Ethyl acetate: Hexane, 20:80 v/v) to elute first $\mathbf{L^2}$ followed by $\mathbf{L^4}$.

2.1.1.2. 2-(1-vinyl-1H-tetrazol-5-yl)pyridine (L^1) and 2-(2-vinyl-2H-tetrazol-5-yl)pyridine(L^3). The same procedure which was used to synthesize $\mathbf{L^2}$ and $\mathbf{L^4}$ was followed with 2-chloro-*N,N*-dimethylehylamine. HCl (1.9 g, 13.6 mmol) to afford $\mathbf{L^1}$ and $\mathbf{L^3}$.

$\mathbf{L^1}$: Off white solid, (0.35 g, yield 30%). M.p. 57–59 °C. Anal. Calc. for $C_8H_7N_5$ (173.17): C, 55.48; H, 4.07; N, 40.44; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.70 (d, 1H, J = 4.8 Hz, pyr-H), 8.46–8.35 (m, 2H, pyr-H + vinyl-H), 7.93 (dt, 1H, J = 8.4, 1.5 Hz, pyr-H), 7.47 (dd, 1H, J = 7.5, 5.7 Hz, pyr-H), 6.26 (d, 1H, J = 15.6, vinyl-H), 5.2 (d, 1H, J = 8.7, Vinyl-H); ^{13}C NMR ($CDCl_3$, 60 MHz): δ 152.9, 149.3, 144.3, 138.1, 127.3, 125.9, 124.3, 102.9 ppm.

$\mathbf{L^3}$: Off white solid, (0.30 g, yield 26%). M.p. 57–58 °C. Anal. Calc. for $C_8H_7N_5$ (173.17): C, 55.48; H, 4.07; N, 40.44; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.82 (d, 1H, J = 4.8 Hz, pyr-H), 8.30 (d, 1H, J = 8.1 Hz, pyr-H), 7.89 (dt, 1H, J = 7.8, 1.8 Hz, pyr-H), 7.70–7.56 (m, 2H, pyr-H + vinyl-H), 7.46–7.40 (m, 1H, pyr-H), 6.42 (dd, 1H, J = 15.9, 1.8 Hz, Vinyl-H), 5.46 (dd, 1H, J = 8.7, 1.5 Hz, Vinyl-H); ^{13}C NMR ($CDCl_3$, 60 MHz): δ 164.5, 150.5, 146.7, 138.3, 127.3, 125.7, 122.35, 102.1.

$\mathbf{L^2}$: white brown solid (0.55 g, yield 34%). M.p. 72–76 °C. Anal. Calc. for $C_{11}H_{16}N_6$ (232.28): C, 56.88; H, 6.94; N, 36.18; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.79 (d, 1H, J = 3.2 Hz, pyr-H), 8.26 (d, 1H, J = 7.8 Hz, pyr-H), 7.87 (dt, 1H, J = 7.8, 1.5 Hz, pyr-H), 7.41 (dd, 1H, J = 7.8, 5.4 Hz, pyr-H), 4.80 (t, 2H, J = 6.9 Hz, CH_2N), 2.53 (t, 2H, J = 6.9 Hz, CH_2), 2.4–2.2 (m, 8H, $N(CH_3)_2 + CH_2$) ppm; ^{13}C NMR ($CDCl_3$, 60 MHz): δ 152.5, 149.5, 144.6, 137.6, 125.4, 122.4, 54.4, 48.2, 43.7, 26.8 ppm.

$\mathbf{L^4}$: white brown solid (0.51 g, yield 32%). M.p. 66–68 °C. Anal. Calc. for $C_{11}H_{16}N_6$ (232.28): C, 56.88; H, 6.94; N, 36.18; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.8 (d, 1H, J = 4.5 Hz, pyr-H), 8.26 (d, 1H, J = 7.8 Hz, pyr-H), 7.88 (t, 1H, J = 6.3 Hz, pyr-H), 7.42 (t, 1H, J = 6.0 Hz, pyr-H), 5.02–4.80 (m, 2H, CH_2N), 2.68 (m, 2H, CH_2), 2.4–2.2 (m, 8H, $N(CH_3)_2 + CH_2$) ppm. ^{13}C-NMR ($CDCl_3$, 60 MHz): δ 164.4, 150.1, 146.6, 138.8, 124.6, 122.1, 56.4, 47.4, 43.5, 26.8 ppm.

2.2. Synthesis of complexes

The appropriate ligand ($\mathbf{L^1}$–$\mathbf{L^4}$) (1.36 mmol) was dissolved in methanol (30 mol) and added to a $MCl_2.H_2O$ (0.68 mmol) methanol solution (10 mol). The resulting pale green to green colored solutions were then heated to reflux for 2–3 h; the solution was left overnight at room temperature and filtered to collect respective precipitate.

$[Ni(L^1)_2]Cl_2$: Dark Green solid (0.12 g, yield 28%) Anal. Calc. for $C_{16}H_{14}Cl_2N_{10}Ni$ (475.95): C, 40.38; H, 2.96; N, 29.43; Found: C, 40.76; H, 2.98; N, 29.96% IR (KBr): ν = 3245, 2945, 1648, 1594, 1496, 1196, 1147, 1023, 835, 785 cm^{-1}. λ_{max} (MeOH) 376 nm, ε = 40 M^{-1} cm^{-1}. Magnetic moment: 3.2 B.M.

$[Ni(L^3)_2]Cl_2$: Pale Green crystals (0.14 g, yield 32%) Anal. Calc. for $C_{16}H_{14}Cl_2N_{10}Ni$ (475.95): C, 40.38; H, 2.96; N, 29.43; Found: C, 40.76; H, 2.98; N, 29.96% IR (KBr): ν = 3245, 2945, 1648, 1594, 1496, 1196, 1147, 1023, 835, 785 cm^{-1}. λ_{max} (MeOH) 388 nm, ε = 38 M^{-1} cm^{-1}. Magnetic moment: 3.2 B.M.

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$[Zn(L^1)_2]Cl_2$: Waxy cream solid (0.17 g, 56%). $C_{16}H_{14}Cl_2N_{10}Zn$(482.64): Calc. C, 39.82; H, 2.92; N, 29.02; Found: C, 26.92; H, 2.63; N, 17.06%. IR (KBr): m = 2945, 2823, 1610, 1565, 1548, 1453, 1058, 1015, 850, 770 cm^{-1}.

$[Zn(L^3)_2]Cl_2$: Waxy orange solid (0.19 g, 58%). $C_{16}H_{14}Cl_2N_{10}Zn$(482.64): Calc. C, 39.82; H, 2.92; N, 29.02; Found: C, 26.92; H, 2.63; N, 17.06%. IR (KBr): m = 2965, 2833, 1612, 1568, 1548, 1453, 1058, 1015, 850, 780 cm^{-1}.

$[Ni(L^2)_2]Cl_2$: Pale Green precipitate (0.12 g, yield 32%) Anal. Calc. for $C_{22}H_{32}Cl_2N_{12}Ni$ (594.17): C, 44.47; H, 5.43; N, 28.29; Found: C, 44.36; H, 5.33; N, 28.96% IR (KBr): v = 3245, 2945, 1648, 1594, 1496, 1196, 1147, 1023, 835, 785 cm^{-1}. λ_{max} (MeOH) 346 nm, ε = 86 M^{-1} cm^{-1}. Magnetic moment: 3.2 B.M.

$[Ni(L^4)_2]Cl_2$: Pale Green precipitate (0.13 g, yield 42%) Anal. Calc. for $C_{22}H_{32}Cl_2N_{12}Ni$ (594.17): C, 44.47; H, 5.43; N, 28.29; Found: C, 44.34; H, 5.32; N, 28.86% IR (KBr): v = 3235, 2925, 1648, 1594, 1496, 1196, 1157, 1023, 835, 785 cm^{-1}. λ_{max} (MeOH) 366 nm, ε = 52 M^{-1} cm^{-1}. Magnetic moment: 3.2 B.M.

$[Zn(L^2)_2]Cl_2$: Waxy cream solid (0.17 g, 56%). $C_{22}H_{32}Cl_2N_{12}Zn$ (600.86): Calc. C, 43.98; H, 5.37; N, 27.97; Found: C, 43.92; H, 5.33; N, 28.36%. IR (KBr): m = 2955, 2823, 1610, 1545, 1548, 1453, 1058, 1015, 850, 770 cm^{-1}.

$[Zn(L^4)_2]Cl_2$: cream solid (0.18 g, 46%). $C_{22}H_{32}Cl_2N_{12}Zn$ (600.86): Calc. C, 43.98; H, 5.37; N, 27.97; Found: C, 43.92; H, 5.33; N, 28.36%. IR (KBr): m = 2965, 2833, 1610, 1545, 1548, 1453, 1058, 1015, 850, 770 cm^{-1}.

2.3. Nematicidal activity

Root knot nematode, *Meloidogyne incognita*, is major plant parasitic nematodes affecting quantity and quality of the crop production in many annual and perennial crops. *Meloidogyne* nematode can develop galls and lesions in the roots, thereby causing stunted growth of the plants. Some of the chemicals can be used to control nematodes [12]. Nematicidal activity of the complexes was carried out on *M. incognita*. Fresh egg masses of *M. incognita* are collected from stock culture maintained on tomato (*Lycopersicon esculentum*) root tissues and kept in water for egg hatching. The egg suspensions were poured on a cotton wool filter paper and incubated at 30 °C to obtain freshly hatched juveniles (J2). Juveniles collected within 48 h were used for screening nematicidal activity of the compounds. The compounds were initially dissolved in dimethyl sulfoxide (DMSO) and then in distilled water to make dilutions of 250, 150, and 50 μg/mL. Experiments were performed under laboratory conditions at 30 °C. About 100 freshly hatched second stage juveniles were suspended in 5 mL of each diluted compound and incubated. Distilled water with nematode larvae was taken as control. The dead nematodes were observed under an inverted binocular microscope. After an incubation of 24 and 48 h, percentage of mortality was calculated. Nematodes were considered dead if they did not move when probed with a fine needle [13].

2.4. DPPH radical scavenging activity

The free radical scavenging activities of the metal complexes were determined by using DPPH free radical scavenging method according to the literature [14]. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis in Table 3. In the spectrophotometric assay the ability to scavenge the stable free radical DPPH is measured by a decrease in the absorbance at 517 nm. Each compound was dissolved in methanol (10 mg/10 mL) and it was used as stock solution.

From the stock solutions, 1 mL of each compound solution with different concentrations (0.25 μg–1.00 μg) was added to the 3 mL of methanolic DPPH (0.004%) solution. After 30 min, the absorbance of the test compounds was taken at 517 nm using UV–vis spectrophotometer. BHT was used as standard, DPPH solution was used as control without the test compounds, and methanol was used as blank. The percentage of scavenging activity of DPPH free radical was measured by using the following formula:

$$\text{Scavenging activity } (\%) = \left[\frac{A_0 - A_i}{A_0}\right] \times 100 \quad (1)$$

where A_0 is the absorbance of the control and A_i is the absorbance of the sample.

2.5. Cytotoxic activity

The human breast carcinoma cell line (MCF-7), human colon carcinoma cell line (COLO 205), and murine microphage cell line (Raw 264.7) were obtained from the National Centre for Cell Science (NCCS),Pune, and grown in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS), amphotericin (3 μg/mL), gentamycin (400 μg/mL), streptomycin (250 μg/mL), and penicillin (250 units/mL) in a carbon dioxide incubator at 5% CO_2. About 700 cells/well were seeded in 96-well plate using culture medium; the viability was tested using trypan blue dye with the help of hemocytometer and 95% of viability was confirmed. After 24 h, the new medium with compounds in the concentrations of 100, 10, and 1 μg/mL was added at respective wells and kept in incubation for 48 h in Table 4. After incubation MTT assay was performed.

2.5.1. MTT assay

After 48 h of the drug treatment the medium was changed again for all groups and 10 μL of MTT (5 mg/mL stock solution) was added and the plates were incubated for an additional 4 h. The medium was discarded and the formazan blue, which was formed in the cells, was dissolved with 50 μL of DMSO. The optical density was measured on a microplate spectrophotometer at a wavelength of 570 nm. The percentage of cell inhibition was calculated by using the following formula [15]:

$$\%\ \text{Growth inhibition} = 100 - (A_0/A_i) \times 100 \quad (2)$$

where A_i is the absorbance of the sample and A_o is the absorbance of the control. IC_{50} values were determined using Graph Pad Prism software.

2.6. DNA cleavage activity

The DNA cleavage activity of metal complexes was monitored by agarose gel electrophoresis. pBR322 plasmid was cultured, isolated, and used as DNA for the experiment. Test samples

(1 mg/mL) were prepared in DMF. 25 μg of the test samples was added to the isolated plasmid and incubated for 2 h at 37 °C. After incubation, 30 μL of plasmid DNA sample mixed with bromophenol blue dye (1:1) was loaded into the electrophoresis chamber wells along with the control DNA, 5 M $FeSO_4$ (treated with DNA), and standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L). Finally, it was loaded on to an agarose gel and electrophoresed at 50 V constant voltage up to 30 min. After the run, gel was removed and stained with 10.01 μg/mL ethidium bromide and image was taken in Versadoc (Biorad) imaging system. The results were compared with standard DNA marker. The same procedure was followed in the presence of H_2O_2 also.

3. Results and discussion

All the Ni(II) complexes were colored, stable, and nonhygroscopic in nature. The complexes are insoluble in common organic solvents but soluble in DMF and DMSO. The elemental analysis showed that the complexes have 1:2 stoichiometry of the type $[M(\mathbf{L}^{1-4})_2]Cl_2$, where **L** stands for singly deprotonated ligands. Molar conductance of the complexes was measured in DMF. The conductance values, which are presented in Table 1, indicate the no electrolytic nature of the complexes [16].

3.1. Determination of the metal content of the complexes

Known amount (0.150 g) of complexes was decomposed with concentrated nitric acid. This process was repeated till the organic part of the complexes got completely lost. The excess nitric acid was expelled by evaporation with concentrated sul-

Table 3 IC_{50} values (μg/mL) of DPPH radical scavenging activity of ligands and their metal complexes.

Compound	IC_{50} (μg/ml)
HL^3	1.28
HL^4	0.43
$Ni(L^1)_2Cl_2$	1.22
$Ni(L^4)_2Cl_2$	1.10
$Zn(L^1)_2Cl_2$	0.72
BHT	0.75

Table 1 Elemental analysis and physical properties of Ni(II) and Zn(II) complexes.

Complex	Molecular formula	Color (% yield)	% Found (cald.)				Molar conductivity (Ohm^{-1} cm^2 mol^{-1})
			C	H	N	M	
$Ni(L^1)Cl_2$	$C_{16}H_{14}Cl_2N_{10}Ni$ (475.95)	Dark green solid	40.38 (40.76)	2.96 (2.98)	29.43 (29.36)	12.33 (12.25)	10
$Ni(L^2)Cl_2$	$C_{22}H_{32}Cl_2N_{12}Ni$ (594.17)	Pale green precipitate	44.47 (44.36)	5.43 (5.53)	28.29 (28.16)	9.88 (9.68)	15
$Ni(L^3)Cl_2$	$C_{16}H_{14}Cl_2N_{10}Ni$ (475.95)	Pale green crystals	40.38 (40.16)	2.96 (2.98)	29.43 (29.36)	12.33 (12.28)	12
$Ni(L^4)Cl_2$	$C_{22}H_{32}Cl_2N_{12}Ni$ (594.17)	Pale green precipitate	44.47 (44.34)	5.43 (5.32)	28.29 (28.86)	9.88 (9.58)	14
$Zn(L^1)Cl_2$	$C_{16}H_{14}Cl_2N_{10}Zn$ (482.64)	Waxy cream solid	39.81 (38.92)	2.92 (2.95)	29.02 (28.78)	13.55 (13.48)	11
$Zn(L^2)Cl_2$	$C_{22}H_{32}Cl_2N_{12}Zn$ (600.86)	Waxy cream solid	43.97 (43.92)	5.37 (5.39)	27.97 (27.36)	10.89 (10.78)	14
$Zn(L^3)Cl_2$	$C_{16}H_{14}Cl_2N_{10}Zn$ (482.64)	Waxy orange solid	39.81 (39.72)	2.92 (2.94)	29.02 (28.78)	13.55 (13.38)	12
$Zn(L^4)Cl_2$	$C_{22}H_{32}Cl_2N_{12}Zn$ (600.86)	Cream solid	43.97 (43.92)	5.37 (5.33)	27.97 (27.36)	10.89 (10.72)	14

Table 2 Electronic, magnetic and ligand field parameters of the pyridyl-tetrazole Ni(II) complexes.

Compound	Absorption maxima (cm^{-1})	Tentative assignments	Magnetic moment (B.M)	ν_2/ν_1	10 Dq (cm^{-1})	B (cm^{-1})	β	LFSE (kJ mol^{-1})
$[Ni(L^1)_2]Cl_2$	8532 15,200 24,900	$3A_2g(F) \rightarrow 3T_2g(F)$ (v1) $3A_2g(F) \rightarrow 3T_1g(F)$ (v2) $3A_2g(F) \rightarrow 3T_1g(P)$ (v3)	3.12	1.78	8532	966	0.92	122.50
$[Ni(L^2)_2]Cl_2$	8787 16,000 24,390	$3A_2g(F) \rightarrow 3T_2g(F)$ (v1) $3A_2g(F) \rightarrow 3T_1g(F)$ (v2) $3A_2g(F) \rightarrow 3T_1g(P)$ (v3)	3.18	1.82	8787	935	0.89	126.19
$[Ni(L^3)_2]Cl_2$	8313 14,705 24,390	$3A_2g(F) \rightarrow 3T_2g(F)$ (v1) $3A_2g(F) \rightarrow 3T_1g(F)$ (v2) $3A_2g(F) \rightarrow 3T_1g(P)$ (v3)	3.25	1.76	8313	943	0.90	119.40
$[Ni(L^4)_2]Cl_2$	8628 16,313 24,570	$3A_2g(F) \rightarrow 3T_2g(F)$ (v1) $3A_2g(F) \rightarrow 3T_1g(F)$ (v2) $3A_2g(F) \rightarrow 3T_1g(P)$ (v3)	2.91	1.89	8628	999	0.95	123.92

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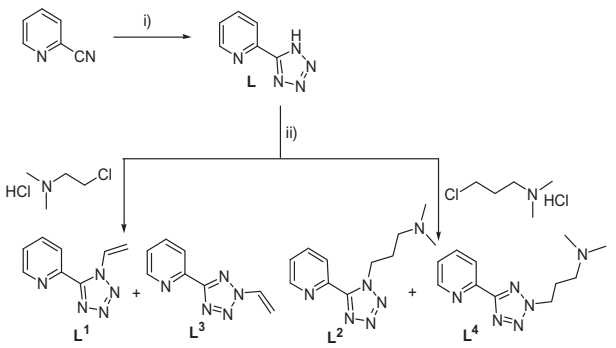
Table 4 IC_{50} values (μg/mL) of cytotoxic activity of ligands and their metal complexes.

Compound	Raw	MCF-7	COLO 205
$\mathbf{L^1}$	46.6	24.6	20.2
$\mathbf{L^2}$	56.2	34.0	39.4
$\mathbf{L^3}$	52.6	29.1	15.3
$\mathbf{L^4}$	47.0	30.2	68.2
$Ni(\mathbf{L^1})_2Cl_2$	223.8	67.2	54.6
$Zn(\mathbf{L^1})_2Cl_2$	44.2	40.1	54.8
$Ni(\mathbf{L^3})_2Cl_2$	36.4	26.6	60.9
$Zn(\mathbf{L^3})_2Cl_2$	33.2	23.2	42.5
$Ni(\mathbf{L^2})_2Cl_2$	54.2	36.8	71.2
$Zn(\mathbf{L^2})_2Cl_2$	33.2	22.6	42.1
$Ni(\mathbf{L^4})_2Cl_2$	58.5	35.8	49.8
$Zn(\mathbf{L^4})_2Cl_2$	44.6	39.4	53.6



Scheme 1 Synthetic route for ligands L^1–$\mathbf{L^4}$, reaction conditions (i) NaN_3, LiCl, NH_4Cl, DMF, reflux 10 h; (ii) K_2CO_3, DMF, 70 °C 24 h.

furic acid. The Ni(II) and Zn(II) contents of the complexes were determined as per the procedure available in the literature [17].

3.2. Spectral data

Ligand (L) on treatment with 2-chloro-*N,N*-dimethylehylamine. HCl in basic medium afforded regio iosmers $\mathbf{L^1}$, $\mathbf{L^3}$ by the elimination of the group $NH(CH_3)_2$ of the reagent used and on treatment of **L** with 3-chloro-*N,N*-dimethyl propan-1-amine. HCl afforded $\mathbf{L^2}$, $\mathbf{L^4}$ by alkylation at either the 1-N or 2-N positions (Scheme 1). The structures for the isomers are readily assigned by their ^{13}C NMR and ^1H NMR spectra. The chemical shift values for the quaternary Carbon of the tetrazole ring appeared in range $\delta \sim 152$ ppm in the 1-N-isomers and at $\delta \sim 164$ ppm in 2-N-isomers. The ^1H NMR spectra of $\mathbf{L^1}$–$\mathbf{L^4}$ showed separately four signals corresponding to pyridyl protons. A multiplet is observed for the six protons of $N(CH_3)_2$ & middle CH_2 in the spectra of $\mathbf{L^2}$ and $\mathbf{L^4}$ at 2.4–2.2 ppm. The alkene protons of $\mathbf{L^1}$ and $\mathbf{L^3}$ adjacent to the tetrazole ring appeared at 8.35, 6.26, 5.20 ppm in Fig. 1 and 7.60, 6.42, 5.46 in Fig. 2. The I.R spectra of ligands ($\mathbf{L^2}$ and $\mathbf{L^4}$) show a broad band in range 1190–1058 cm^{-1} corresponding to – $N(CH_3)_2$, and peaks at 1650–1500 cm^{-1}. The presence of a signal at 2218 cm^{-1} in the IR spectrum indicated an azide bond (N–N or *N,N* band) in Fig. 3 [18]. Two similar peaks around 1150–900 cm^{-1} correspond to the tetrazole group [19]. The ligands ($\mathbf{L^1}$–$\mathbf{L^4}$) are treated with $NiCl_2.2H_2O$ and $ZnCl_2.2H_2O$ salt, in methanol at reflux temperature under N_2 atmosphere for 2–3 h. All reactions were carried out using a 1:2 metal: ligand stoichiometry ratio to give corresponding complexes (Scheme 2). The formation of coordination bonds between the tetrazole ring and Ni(II) and Zn(II) is confirmed by observing the IR frequencies of the tetrazole ring. The ligands $\mathbf{L^1}$–$\mathbf{L^4}$ showed characteristic absorption bands (IR) at 1630–1570 cm^{-1} which are shifted to lower frequencies in all

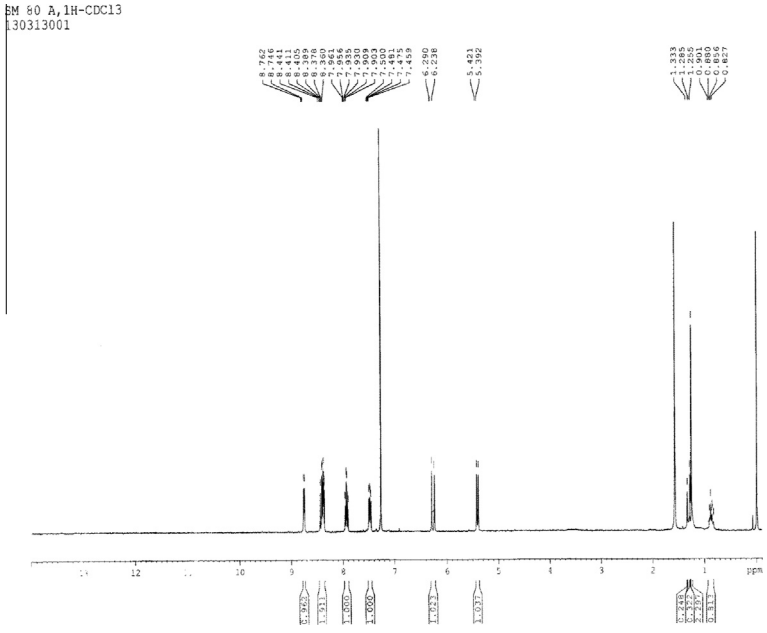


Figure 1 ^1H NMR spectrum of 2-[5-(pyridin-2-yl)-1H-tetrazol-1-yl]propyl-N,N-dimethylamine($\mathbf{L^1}$) in $CDCl_3$ solvent.

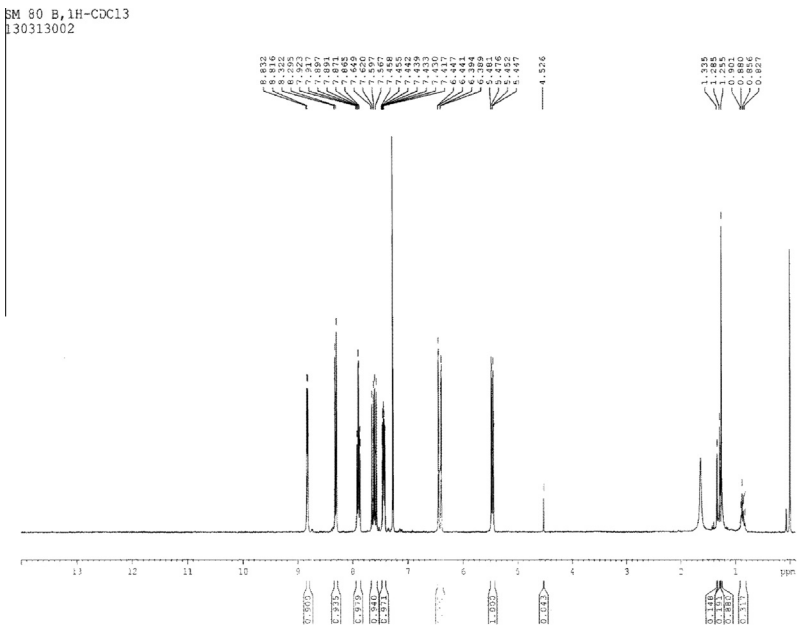


Figure 2 ^{1}H NMR spectrum of 2-[5-(pyridin-2-yl)-1H-tetrazol-2-yl]propyl-N,N-dimethylamine($\mathbf{L^3}$) in $CDCl_3$ solvent.

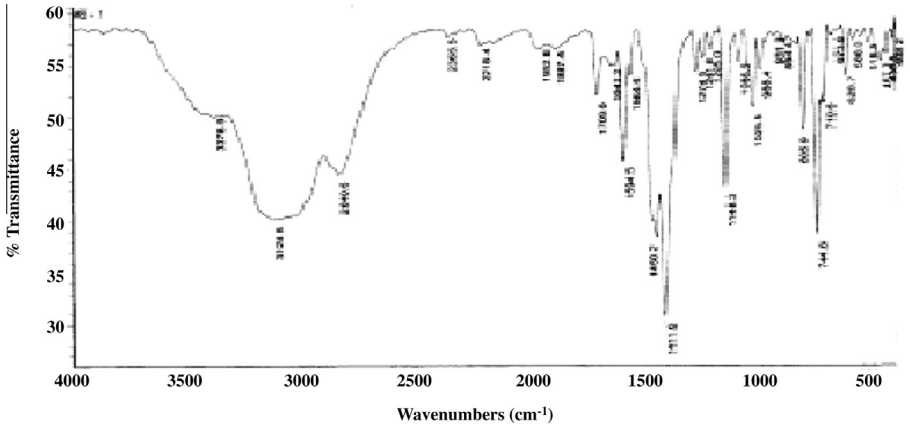
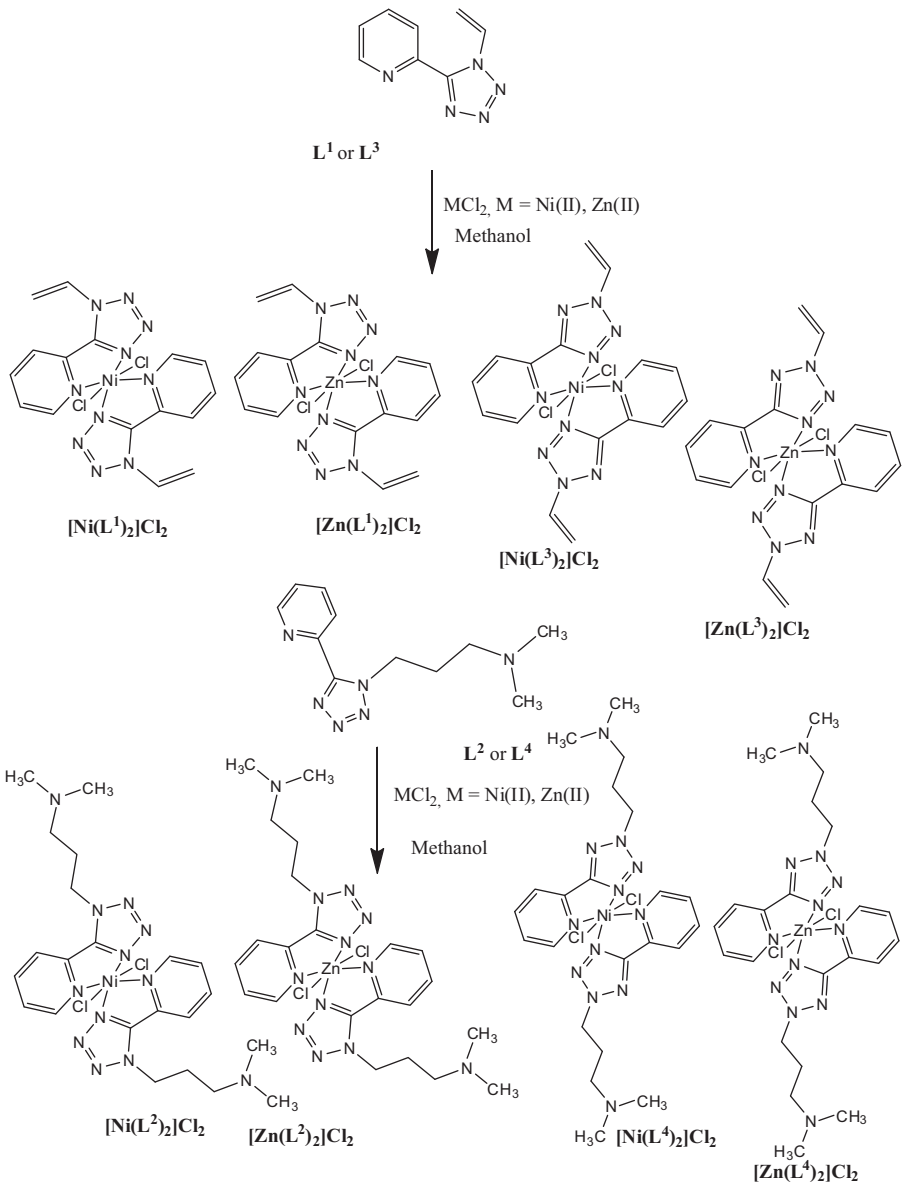


Figure 3 FT-IR spectra of 2-[5-(pyridin-2-yl)-1H-tetrazol-1-yl]propyl-N,N-dimethylamine($\mathbf{L^2}$) in KBr.

Ni(II) and Zn(II) complexes. Additional peaks around 1340–1200 cm^{-1} and 800–600 cm^{-1} appeared due to the coordination of the pyridine ring with Ni(II) and Zn(II) ions. The elemental analysis of the obtained complexes showed that all the complexes obtained are in 1:2 (metal: ligand) compositions. All Nickel complexes have magnetic moments value ranging 3.2 B.M expected for a d8 system with one unpaired electron [20] in Ni(II) complexes.

3.3. Electronic spectra and magnetic moments

The electronic absorption spectra of the isomeric pyridyl tetrazole metal complexes in solid state were recorded at room temperature and the band positions of the absorption maxima, band assignments, ligand field parameters, and magnetic moment values are listed in Table 2. The electronic spectra of Ni(II) complexes displayed three absorption bands in the range 8000–9000, 14,000–16,000, and 20,000–24,000 cm^{-1}. Thus, these bands may be assigned to the three spin allowed transitions $3A_2g(F) \rightarrow 3T_2g(F)$ (ν_1), $3A_2g(F) \rightarrow 3T_1g(F)$ (ν_2), and $3A_2g(F) \rightarrow 3T_1g(P)$ (ν_3),respectively, characteristic of octahedral geometry. The values of transition ratio [ν_2/ν_1] and β lie in the range of 1.70–1.80 and 0.89–0.95, respectively, providing further evidence for octahedral geometry of Ni(II) complexes [21]. The β values for the complexes are lower than the free ion value, thereby indicating orbital overlap and delocalization of d-orbitals.The β-values obtained are less than unity suggesting the covalent character of the metal–ligand bonds. All Ni(II) complexes are paramagnetic and the magnetic movement values at room temperature are in the range of 2.91–3.25 B.M which is well agreed with the reported octahedral Ni(II) complexes [22]. All Zn(II) complexes showed two bands around 25,000 and 30,000 cm^{-1} and are attributed to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively. Zn(II)



Scheme 2 Synthesis of Ni and Zn complexes with pyridyl-tetrazole ligands ($\mathbf{L^1}$–$\mathbf{L^4}$).

complexes that are in d^{10} configuration are diamagnetic and do not show any d–d transitions.

3.3.1. DNA cleavage studies

The interaction of plasmid pBR322 DNA with Ni(II) and Zn(II) complexes was studied using gel electrophoresis in the presence and absence of oxidizing agent H_2O_2. DNA cleavage was achieved by monitoring the gel electrophoresis for naturally occurring, covalently closed circular form (Form I) transition to the nicked circular (Form II) and linear forms (Form III).

When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the super coil form (Form I), slower migration will be observed for nicked circular form (Form II) and linear form occurred between the super coiled and nicked circular forms. The gel electrophoresis pictures are shown in Fig. 4. In the absence of H_2O_2, control DNA (In Fig. 4a and b. Lane control) does not show any activity. $FeSO_4$ was used as standard, disappearance of bands was observed in all the complexes except $[Ni(\mathbf{L^3})_2]Cl_2$ and $[Zn(\mathbf{L^3})_2]$; $[Zn(\mathbf{L^4})_2]Cl_2$ complexes indicate the complete DNA cleavage activity. In the case of $[Ni(\mathbf{L^3})_2]$-Cl_2, $[Zn(\mathbf{L^3})_2]$, and $[Zn(\mathbf{L^4})_2]Cl_2$ complexes a decrease in the intensity of bands was observed compared to the control. This is probably due to the partial cleavage of the DNA. The DNA cleavage activity of the complexes in the presence of H_2O_2 may be due to the reaction of hydroxy radicals with DNA. The general oxidative mechanisms of the DNA cleavage studies were reported by several research groups [23–25]. Many literature reports infer that the compound was to cleave the DNA; it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome [26].

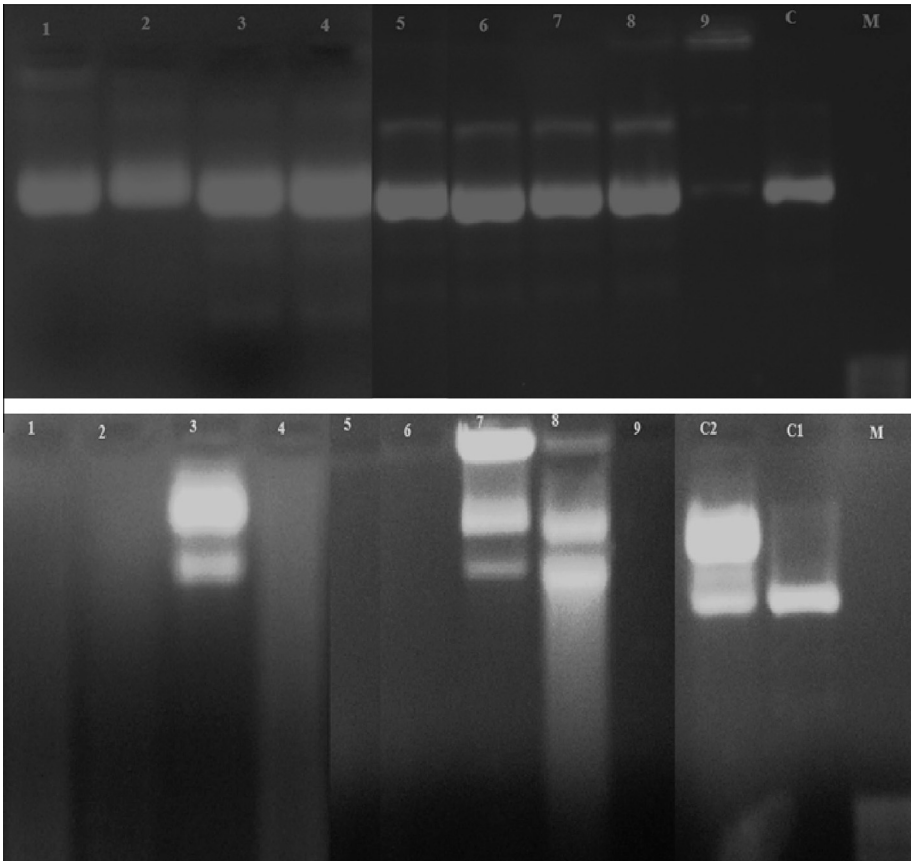


Figure 4 Gel electrophoresis photograph of metal complexes. (a) Gel electrophoresis photograph showing the effects of metal complexes on Pbr322 DNA: lane 1, DNA + $[Ni(\mathbf{L^1})_2]$; lane 2, DNA + $[Ni(\mathbf{L^2})_2]Cl_2$; lane 3, DNA + $[Ni(L^3)_2]Cl_2$; lane 4, DNA + $[Ni(L^4)_2]Cl_2$; lane 5, DNA + $[Zn(L^1)_2]Cl_2$; lane 6, DNA + $[Zn(\mathbf{L^2})_2]$; lane 7, DNA + $[Zn(\mathbf{L^3})_2]$; lane 8, DNA + $[Zn(L^4)_2]Cl_2$; lane 9, DNA + $FeSO_4$; lane C, DNA alone. (b) Gel electrophoresis photograph showing the effects of metal complexes on pBR322-DNA in the presence of H_2O_2: lane 1, DNA + $[Ni(L^1)_2]$ + H_2O_2; lane 2, DNA + $[Ni(L^2)_2]Cl_2$ + H_2O_2; lane 3, DNA + $[Ni(\mathbf{L^3})2]Cl_2$ + H_2O_2; lane 4, DNA + $[Ni(\mathbf{L^4})_2]$ + H_2O_2; lane 5, DNA + $[Zn(L^1)_2]Cl_2$ + H_2O_2; lane 6, DNA + $[Zn(L^2)_2]$ + H_2O_2; lane 7, DNA + $[Zn(L^3)_2]$ + H_2O_2; lane 8, DNA + $[Zn(\mathbf{L^4})_2]Cl_2$ + H_2O_2; lane 9, DNA + $FeSO_4$ + H_2O_2; lane C2, DNA + H_2O_2; lane C1, DNA alone.

4. Conclusions

Ni(II) and Zn(II) complexes have been synthesized using bidentate isomeric pyridyl tetrazole ligands and characterized by various analytical and spectral data. Based on the electronic spectra, magnetic moment, and elemental analysis data, octahedral geometry was proposed for Ni(II) and Zn(II) complexes. The nematicidal activity of metal complexes revealed that $[Ni(\mathbf{L^3})_2]Cl_2$ and $[Zn(\mathbf{L^3})_2]Cl_2$ complexes showed moderate activity. $[Zn(\mathbf{L^4})_2]Cl_2$ complex exhibited greater antioxidant activity compared to the remaining metal complexes. All metal complexes exhibited considerable cytotoxic activity against Raw, MCF-7, and COLO 205 cell lines. The DNA cleavage studies of metal complexes showed more prominent activity in the presence of H_2O_2 compared to that in the absence of H_2O_2.

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